Rapid Evolution of Metal Resistance in a Benthic Oligochaete Inhabiting a Metal-polluted Site

P. L. KLERKS* AND J. S. LEVINTON

Department of Ecology & Evolution, State University of New York, Stony Brook, New York 11794

Abstract. We identified a case of very rapid evolution of resistance in a common freshwater benthic invertebrate, to sediment with extremely high levels of cadmium and nickel. *Limnodrilus hoffmeisteri* from metal-polluted sites in Foundry Cove (New York) was significantly more resistant than conspecifics from a nearby control site, to both metal-rich natural sediment and metal-spiked water. Resistance differences were also found among sites within Foundry Cove. The elevated resistance in Foundry Cove worms was genetically determined, as it was still present after two generations in clean sediment. Resistance had evolved rapidly (within 30 years). A laboratory selection experiment and estimates of the heritability of this resistance in *L. hoffmeisteri* from the control site, indicated that the resistance could have evolved in 1 to 4 generations. The laboratory selection resulted in a large increase in resistance after two generations of selection, while we demonstrated that most of the phenotypic variation was additive genetic; heritability estimates ranged from 0.59 to 1.08.

Introduction

Environmental pollutants may exert strong selective pressures on natural populations, offering unique opportunities for studying natural selection. The selective agent, selection strength, and the timing of the selection process are often known and quantifiable, while the selective pressures may be so strong that effects become evident rapidly. In addition, the evolution of resistance

to pollutants can illustrate the processes involved in early stages of speciation (Macnair, 1987). But considerations of a less theoretical nature also underscore the importance of investigating the evolution of resistance. If populations inhabiting polluted sites commonly evolve a resistance to the toxicants present, long-term effects of pollutants on natural populations will be very different from those predicted from bioassays (Levinton, 1980).

The evolution of resistance to metals is well documented for plants (Antonovics *et al.*, 1971; Klerks and Weis, 1987; and Macnair, 1987), but very little evidence is available demonstrating metal adaptation in natural populations of animals (Klerks and Weis, 1987). However, it is known that genetic variation for metal resistance is present in several populations of aquatic animals (*e.g.*, Lavie and Nevo, 1982; Nevo *et al.*, 1984; and Lavie and Nevo, 1986). And an elevated metal resistance is reported for many populations of aquatic animals (Klerks and Weis, 1987). But the presence of a genetic basis for these resistance differences among populations from differently polluted environments is confirmed only by Brown (1976) for a population of isopods inhabiting a metal-polluted river in S.W. England. In addition, there is some evidence that the increased resistance in the polychaete *Nereis diversicolor* from several metal-polluted rivers in S.W. England (Bryan and Hummerstone, 1971, 1973; Bryan, 1976) might be genetically determined, as de-acclimation of copper-resistant worms did not result in a reduced resistance (Bryan and Hummerstone, 1971; Bryan and Gibbs, 1983).

Most polluted areas contain many different toxicants. This complexity hinders an investigation into the evolution of resistance, as this makes it difficult to pinpoint the selective force(s). An unusual situation exists in Foundry Cove, New York (a freshwater bay on the Hudson River). This tidal cove and marsh has elevated levels of

a very limited set of pollutants. Foundry Cove received wastewater rich in Cd, Ni, and (at times) Co from a battery factory, during the period 1953–1971 (Resource Engineering, 1983). Sediment Cd levels as high as 50,000 μg Cd per g dry sediment were reported for 1975 (Kneip and Hazen, 1979). More recent findings showed decreased Cd concentrations in the surface layer of the sediment, though much sediment still contains over 500 μg/g Cd. An extremely high Cd level (225,000 μg/g) was encountered in 1983 close to the original outfall site at a depth of 10–20 cm (Knutson *et al.*, 1987). Concentrations of Cd, Ni, and Co are highly correlated, and the three metals occur in molar ratios of 18:20:1, respectively (Knutson *et al.*, 1987). Benthic macrofauna taken from this sediment and allowed to exclude their gut contents, have elevated body burdens of both Cd and Ni (Klerks, 1987). This demonstrates that the Cd and Ni in Foundry Cove sediment are bioavailable. Nevertheless, organisms are present in this sediment. Overall densities of macrobenthos in Foundry sediment are similar to those of the control area, though there is evidence for a reduction of the taxonomic diversity at the most polluted site (Klerks and Levinton, 1989).

We investigated the evolution of resistance to a combination of Cd, Ni, and Co in the deposit feeding annelid *L. hoffmeisteri*. This tubificid oligochaete is the most common macrobenthic species at both Foundry Cove and a nearby control site (Klerks, 1987). The evolution of metal resistance was approached in two ways. To determine if *L. hoffmeisteri* from Foundry Cove was genetically adapted to the metals, we compared the resistance of these worms and their offspring to the resistance in conspecifics from the control site. Our results showed that *L. hoffmeisteri* from Foundry Cove was genetically adapted to the pollutants. To find out how likely it was that such an adaptation would occur again and to confirm our evidence for a very rapid evolution of resistance, we determined the heritability of the metal resistance in the control population and performed an experiment in which control worms were selected for an increased resistance. These experiments showed that the control population had the potential for a rapid evolution of resistance to a combination of Cd, Ni, and Co.

Materials and Methods

Animal collections and laboratory cultures

Both *L. hoffmeisteri* and sediment were collected by Ekman grab in either Foundry Cove (near Cold Spring, New York, and 87 km upriver from the southern tip of Manhattan) or the control area (South Cove, about 2 km south of Foundry Cove). Worms were sorted from the >500 μm fraction of sediment. Laboratory cultures were set up in polystyrene dishes, with a 1-cm layer of sedi-

ment and 9 cm of continuously aerated Hudson River water. The sediments for the cultures were collected from the same sites as the animals, sieved (to <500 μm), boiled, washed with GF-C filtered Hudson River water, frozen, and thawed shortly before use. Hudson River water was collected near the control area and filtered with a GF-C glassfiber filter prior to use. Ground fishfood flakes were added to the cultures once per week unless previously added food was still present. Cultures were kept at 24°C under a 13 h light/11 h dark cycle.

To obtain the second generation of unexposed offspring, Foundry Cove worms were cultured in sediment from the control area (containing 19 μg/g Cd), for two generations. Newborn juveniles were separated from the adults approximately monthly, to keep the different generations apart.

L. hoffmeisteri is a simultaneous hermaphrodite, reproducing sexually by crossfertilization, though uniparental reproduction has been reported (Gavrilov, 1931). Autotomy and fragmentation followed by regeneration are common, but do not result in additional individuals (Kennedy, 1966).

Resistance comparisons

Experiments comparing the toxicity of metal-rich sediments to *L. hoffmeisteri* from Foundry Cove and the control site were done in glass beakers (10 cm diameter), containing a 1-cm layer of sediment and 9 cm of aerated overlying water. Water and sediment were treated similarly as for the cultures, except that these sediments had been sieved to a <250 μm size to facilitate detection of newborn juveniles. These bioassays were performed with natural sediments from Foundry Cove and the control area. The experiments were started with ten adult worms per replicate and three replicates per group. Ground fishfood flakes (0.05 g) were added at the beginning of the experiment and after 14 days. The number of survivors (and newborn juveniles) were determined after 28 days. The first experiment using this experimental procedure was performed with worms collected from Foundry Cove (from a site with approximately 7000 μg Cd per g dry sediment) and from the control area (19 μg/g Cd). The same procedure was used in a second experiment to investigate the presence of resistance differences between worms collected from the control area, worms from Foundry Cove, and the second generation offspring of Foundry Cove worms.

Metal concentrations of sediments were determined by flame atomic absorption spectrophotometry. Samples were homogenized, dried at 70°C, and ground to a powder. Small aliquots (<1 g) were filtered after refluxing with 7.5 ml each of nitric and hydrochloric acids (reagent grade) for 5 h at 120°C. The filtrate was brought up to 50

ml with distilled water and analyzed with an Instrument Laboratory Model 157 spectrophotometer.

A second type of bioassay involved the determination of survival times in metal-spiked water. Mature *L. hoffmeisteri* were exposed to a solution containing 8.9 μM Cd, 10.2 μM Ni, and 0.5 μM Co (nominal concentrations using chloride salts), in soft reconstituted fresh water (ASTM, 1980). (The specific metal concentrations were chosen arbitrarily, though the ratios for the three metals were the same as in Foundry Cove sediments). The water was aerated prior to use and animals were acclimated overnight to this water. Both acclimation and exposure took place in plastic Petri dishes (100×15 mm) with 40 ml reconstituted water or metal solution at an initial density of 6 worms per dish. Animals were transferred to new dishes (the 6 worms from an acclimation dish were transferred together) after the acclimation period. At time zero, 0.4 ml of a solution containing 100 times the experimental metal concentrations was added to 39.6 ml water. Experimental control animals were treated similarly as the exposed animals except that no metal stock solution was added. The animals were not fed during either acclimation or exposure. Survival was determined every hour. The experiment was terminated when all the exposed animals had died. Actual metal concentrations in solution were not determined, since the purpose of these experiments was entirely comparative. The first experiment using this type of bioassay was performed with worms from the control area and from three differently polluted sites in Foundry Cove (7 replicate dishes with 6 worms each per group). Subsequent experiments using this procedure were performed on groups of worms from selection lines (usually 10 replicate dishes with 6 worms each per group, see below) and on control area worms for the heritability determinations (with 1 to 6 worms per dish, see below).

Selection experiments

The starting population was initiated by combining control area *L. hoffmeisteri* from several laboratory cultures. Worms from the different cultures were divided equally over four lines (three selection lines and a control line), resulting in 441 juveniles for each of the lines. In selecting the first generation, 200 adults from each of the three selection lines were exposed to Cd-rich sediment (approximately 50,000 $\mu g/g$ Cd) until 80 to 90% of the worms had died. The surviving adults were then transferred to reproduce in dishes with control area sediment. The ultimate number of survivors was determined after three months and these were transferred again to clean sediment. The latter was done to exclude juveniles born from mating occurring prior to the final selection of the parent generation, since spermatophores may remain in

spermatheca for up to six weeks (Kennedy, 1966). The next generation was later started with 500 of their offspring per line, in clean sediment.

The selection procedure for subsequent generations was modified to achieve a shorter time between generations. Worms with not fully developed gonads were exposed to a combination of 8.9 μM Cd, 10.2 μM Ni, and 0.5 μM Co in soft reconstituted fresh water for a duration aimed at an ultimate survival of 25%. Initial survivors were transferred to culture dishes with clean sediment. These worms were checked monthly to determine ultimate survival and to remove 500 juveniles (per line) for the next generation.

For the control line, the number of adults was reduced to achieve a similar inbreeding effect as in the selected lines, using the harmonic mean of the population sizes in successive generations to determine the mean effective population size and the mean rate of inbreeding (Falconer, 1981).

The selection process was continued for three generations. The proportion of the population selected ranged from 7.5 to 29% (mean: 14%).

The resistance of each line in each generation was quantified by determining survival times in the above-described bioassay with Cd, Ni, and Co in reconstituted water. Survival times were generally determined on 60 mature worms per line. For generation zero, only 60 mature worms were available to quantify survival times: 30 for the combined selection lines and 30 for the control line.

Heritability determinations

Control area worms that did not show any gonadal development were placed in 4-oz. (118 ml) plastic beakers (160 worms, 2 per beaker), containing a 1-cm layer of sediment and 2 cm of water (GF-C filtered Hudson River water) that was aerated continuously. Water was added regularly to maintain initial water levels. The beakers were sorted monthly and juveniles were transferred to separate beakers (up to 6 worms per dish and a maximum of 18 offspring per pair of adults). Some ground fishfood was added once the previously added food had disappeared. About half the pairs had produced offspring after 3–4 months. Resistance was again quantified as survival times in 8.9 μM Cd, 10.2 μM Ni, and 0.5 μM Co in water, using mature worms.

Heritability estimates were obtained using resemblances in survival times among relatives (Falconer, 1981), on log-transformed survival times. Estimates were obtained from the regressions of "mean offspring survival time" on "midparent survival time" (both on unweighted data, as well as the data weighted for the number of offspring per family), and "offspring survival time"
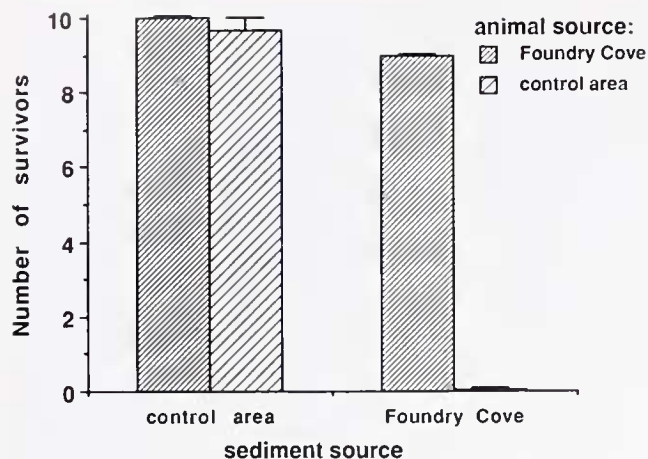
Figure 1. Numbers of *Limnodrilus hoffmeisteri* from Foundry Cove and the control area, surviving a 28-day exposure to sediments from each location. Mean and S.E._mean are shown, three replicates per group, each replicate started with 10 individuals.

on "parent survival time." The heritability (h^2) was computed as the regression coefficient (b) of the midparent-offspring regression, and $h^2 = 2b$ for the parent-offspring regression. The weighting for family size was performed according to Falconer (1981).

An additional estimate of the heritability was obtained from the results of the selection process. This "realized heritability" was determined as the cumulative response, divided by the cumulative selection differential (Falconer, 1981).

Results

Resistance comparisons

The first step in our investigation into the evolution of resistance to the metal-pollution of Foundry Cove was aimed at determining if *L. hoffmeisteri* from this area had an elevated resistance. These Foundry Cove worms were indeed more resistant to metal-rich sediment than their conspecifics from the control area (Fig. 1). The difference in survival was highly significant for animal source and animal × sediment interaction (both $P < 0.001$) in an ANOVA on the square root of the number of survivors.

An exposure to Cd, Ni, and Co in water revealed the presence of resistance differences among worms collected at sites with different metal levels in the sediment (Fig. 2). As before, worms from the routine collecting site in Foundry Cove (with about 7000 µg Cd per g sediment) were significantly more resistant than the ones from the control area [in unplanned comparisons among pairs of means using the Games and Howell method (Sokal and Rohlf, 1981) since variances were heterogeneous].

Within Foundry Cove, the animals from a relatively clean part of the cove were significantly less resistant than those from the routine collecting site. Though the resistance of worms from the most polluted site in Foundry Cove approached that of the worms from the routine collecting site, their resistance did not differ significantly from that of the worms from any of the other sites (probably due to the large variation in resistance among worms from the most polluted site).

As the individuals from Foundry Cove used in these experiments had previously encountered metal-rich sediment, the elevated resistance could have been due to physiological acclimation, genetic adaptation, or both. To investigate the presence of a genetic component in the resistance differences, resistance in the second generation offspring was compared to that of the worms from the control area and Foundry Cove. The results are shown in Figure 3. Overall, control area worms were significantly less resistant than both groups of Foundry Cove worms [in a multiple t-test (LSD) at $\alpha = 0.05$ on the square root of the number of survivors]. For the separate Cd levels, no statistically significant differences were evident at the low (430 µg/g Cd) and high (63,000 and 90,000 µg/g) Cd levels. Survival at 22,400 and 31,600 µg/g Cd showed the same pattern as for the overall data, while at 44,700 µg/g Cd the worms collected from Foundry Cove survived significantly better than the two other groups. These results demonstrated that *L. hoffmeisteri* from Foundry Cove was genetically adapted to the Cd-rich sediment. Overall, no significant difference in resistance was detected between the worms from
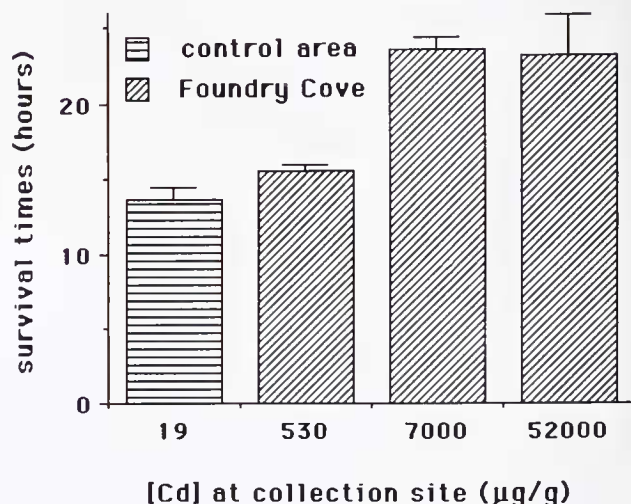

Figure 2. Survival times of *Limnodrilus hoffmeisteri* from the control area and three differently polluted sites in Foundry Cove, when exposed to a mixture of Cd, Ni, and Co in water (respect. 8.9, 10.2, and 0.5 µM). Mean and S.E._mean are shown, 7 replicates (with 6 worms each) per group.
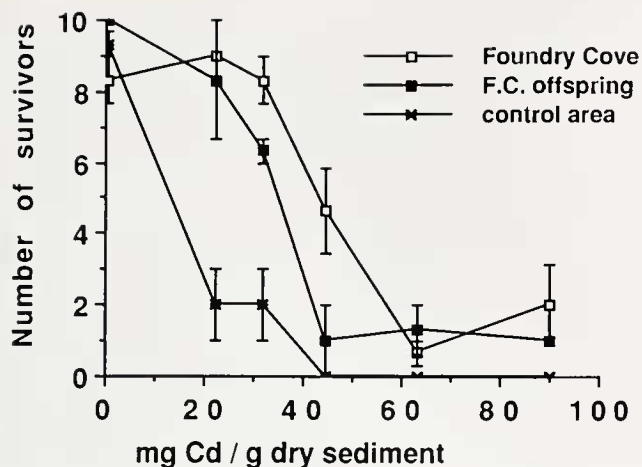
Figure 3. Numbers of field-collected *Limnodrilus hoffmeisteri* from Foundry Cove and the control area, and second generation offspring of Foundry Cove worms surviving a 28-day exposure to sediments with different Cd levels. Lines connect means, vertical lines represent S.E.$_{mean}$. Three replicates per group, each replicate was started with 10 individuals.

Foundry Cove and their offspring that had never been exposed to metal-rich sediment.

Selection experiments

L. hoffmeisteri from the control area was selected for resistance to a combination of Cd, Ni, and Co in water, to determine the potential for the evolution of resistance in this population. No response to the selection was evident after one generation of selection but later generations showed a significantly higher resistance than the control line (Fig. 4). After three generations of selection, the difference in resistance between the selected lines and the control line averaged 66% of the difference in resistance between field-collected Foundry Cove worms and those from the nearby control site.

Heritability determinations

The potential for adaptation to metals in the control population was also assessed by determining the heritability of metal-resistance in this population. Regressions of the average resistance of offspring on the average resistance of their parents resulted in heritability estimates (±S.E.) of 0.93 ± 0.12 (n = 28) and 0.92 ± 0.12 (n = 28) (the latter value for data weighted for the number of offspring per family). Single parent-offspring regression resulted in an heritability estimate of 1.08 ± 0.10 (n = 455).

The result from the selection experiment translated into a realized heritability of 0.59 ± 0.14 (n = 11), somewhat lower than the heritability estimates given above, but still indicating the presence of much heritable genetic variation.

Discussion

Limnodrilus hoffmeisteri from Foundry Cove was much more resistant to both Foundry Cove sediment with high Cd, Ni, and Co levels and water spiked with these metals, than conspecifics from the control site. The elevated resistance remained after two generations in clean sediment. This demonstrated that *L. hoffmeisteri* from metal-polluted Foundry Cove was genetically adapted to the combination of cadmium, nickel, and cobalt. The second generation offspring (that had never encountered metal-rich sediment previously) were not less resistant than the worms that were collected in Foundry Cove. Therefore, we have no evidence for the presence of an environmental component in the resistance differences or for a decrease in resistance in the absence of a continued selection pressure. The occurrence of a genetic adaptation to metals in a population of oligochaetes inhabiting a metal polluted site agrees with findings for a population of isopods (Brown, 1976). While several other studies did not detect resistance differences among populations from differently polluted environments, this absence of resistance differences could be due to the possibility that those pollutants in question failed to affect these populations negatively (Klerks and Weis, 1987).

The metal adaptation in Foundry Cove worms was demonstrated about 30 years after the onset of the pollution at this site. This showed that metal resistance can
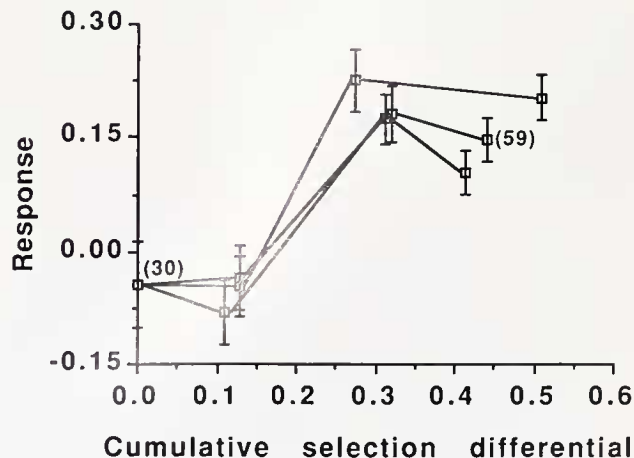

Figure 4. Selection response as a function of the cumulated selection differential, in *Limnodrilus hoffmeisteri* exposed to a mixture of Cd, Ni, and Co in water (respect. 8.9, 10.2, and 0.5 μM). Lines connect the response means in three selection lines (response determined as the difference in average survival time between a selected line and the control line), while vertical lines represent the 95% confidence interval of this difference. n = 60 for each determination, except where indicated otherwise.

evolve rapidly. Such an extremely rapid evolution of metal resistance was not evident from the metal adaptation in populations in S.W. England mentioned earlier (Bryan and Hummerstone, 1971; Brown, 1976), where the metal pollution has lasted more than several hundred years (ref. in Brown, 1976; Bryan and Gibbs, 1983).

We found a difference in metal resistance between Foundry Cove worms collected from sites (with different metal levels) that were only 200 m apart. This indicated that selection is possibly very strong. But the alternative, that gene-flow is extremely limited in these worms, cannot be ruled out since little is known about the amount of gene-flow in these animals. However, selection for metal resistance in natural populations can be strong enough to maintain resistance differences despite high gene flow (McNeilly, 1968).

We estimated that most of the variation in resistance in the control population is additive genetic variation and constitutes variation on which natural selection can act (h^2 values ranging from 0.6 to 1.0). It is possible that part of the resemblance in resistance between parents and offspring is due to the presence of a common environment (as the offspring spent up to 12 weeks in the dish containing their parents). This could have lead to an overestimation of the heritability. If the resemblance between parents and offspring was entirely due to this common environment, offspring should have resembled the individual parents to the same extent as the midparent. This was clearly not the case since the coefficient for the parent-offspring regression was 58% of the midparent-offspring regression (regression coefficients were 0.54 and 0.93, respectively). Therefore, it is unlikely that more than a minor proportion of the resemblances between parents and their offspring had an environmental basis.

The presence of much heritable variation in metal resistance has been reported for plants (McNeilly and Bradshaw, 1968), but no heritability estimates on populations of animals are available for comparison. The observed rapid evolution of resistance in *L. hoffmeisteri* is consistent with our heritability estimates. Using the conservative estimate for the heritability of 0.6, it takes (theoretically) 38 generations of selection at a fairly low intensity of selection (95% of the population contributing to the next generation), to obtain the resistance-difference observed between Foundry Cove and control area worms. With 40% of the population contributing to the next generation (which seems a realistic value for control area worms in Foundry Cove sediment based on our experiments) the resistance would have evolved in 4 generations, while at a high intensity of selection (¼% of the population contributing to the next generation), one generation of selection would have been sufficient.

The observed rapid evolution of resistance to metals is also consistent with the laboratory selection experiment. After three generations of selection, the response was equivalent to 66% of the resistance difference observed among the natural populations of *L. hoffmeisteri*. A positive response to selection for metal resistance was also obtained by Moraitou-Apostolopoulou *et al.* (1983) for a benthic copepod. In contrast, attempts to select for metal resistance were unsuccessful in flagfish (Rahel, 1981) and daphnids (LeBlanc, 1982).

The main importance of this study is not its demonstration of natural selection; natural selection in wild populations has been documented extensively (Endler, 1987). This study is unusual in that it demonstrated that the response to directional selection in natural populations can be extremely rapid. The spectacular potential of natural selection to change the physiological capabilities of aquatic invertebrate populations should also be realized in the context of predictions of the effects of pollutants on natural ecosystems. Evidence that populations may adapt to environmental pollutants is accumulating. However, it seems unlikely that all species will adapt to pollutants. For example, in the chironomid fly *Tanypus neopunctipennis* we found no difference in resistance between animals from Foundry Cove and the control site. This may relate to the greater gene flow among sites in this species, or the lower metal accumulation (Klerks, 1987). A number of species are missing from the most polluted site in Foundry Cove, similar to the situation in many polluted environments (Klerks and Weis, 1987). Therefore it would be totally unjustified to see the evolution of resistance as a justification for the release of pollutants in natural environments.

Acknowledgments

Literature Cited

Antonovics, J., A. D. Bradshaw, and R. G. Turner. 1971. Heavy metal tolerance in plants. *Adv. Ecol. Res.* 7: 1–85.

ASTM. 1980. *Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians.* ASTM: E729–80.

Brown, B. E. 1976. Observations on the tolerance of the isopod *Asellus meridianus* Rac. to copper and lead. *Water Res.* 10: 555–559.

Bryan, G. W. 1976. Some aspects of heavy metal tolerance in aquatic organisms. Pp. 7–34 in *Effects of Pollutants on Aquatic Organisms,* A. P. M. Lockwood, ed. Cambridge University Press, Cambridge.

Bryan, G. W., and P. E. Gibbs. 1983. Heavy metals in the Fal Estuary, Cornwall: a study of long-term contamination by mining waste and its effects on estuarine organisms. *Occasional Publication, Mar. Biol. Assoc. U.K.* 2: 1–112.

Bryan, G. W., and L. G. Hummerstone. 1971. Adaptation of the poly-

chaete *Nereis diversicolor* to sediments containing high concentrations of heavy metals. I. General observations and adaptation to copper. *J. Mar. Biol. Assoc. U. K.* **51**: 845–863.

Bryan, G. W., and L. G. Hummerstone. 1973. Adaptation of the polychaete *Nereis diversicolor* to estuarine sediments containing high concentrations of zinc and cadmium. *J. Mar. Biol. Assoc. U. K.* **53**: 839–857.

Endler, J. 1987. *Natural Selection in the Wild.* Princeton University Press, Princeton.

Falconer, D. S. 1981. *Introduction to Quantitative Genetics.* Longman, London.

Gavrilov, K. 1931. Selbstbefruchtung bei *Limnodrilus. Biol. Zbl.* **51**: 199–206.

Kennedy, C. R. 1966. The life history of *Limnodrilus hoffmeisteri* Clap. (Oligochaeta: Tubificidae) and its adaptive significance. *Oikos* **17**: 158–168.

Klerks, P. L. 1987. *Adaptation to metals in benthic macrofauna.* Ph.D. Thesis, State University of New York, Stony Brook.

Klerks, P. L., and J. S. Levinton. 1989. Effects of heavy metals in a polluted aquatic ecosystem. Pp. 41–67 in *Ecotoxicology: Problems and Approaches.* S. A. Levin, M. A. Harwell, J. R. Kelly, and K. D. Kimball, eds. Springer Verlag, New York.

Klerks, P. L., and J. S. Weis. 1987. Genetic adaptation to heavy metals in aquatic organisms: a review. *Environ. Pollut.* **45**: 173–205.

Kneip, T. J., and R. E. Hazen. 1979. Deposit and mobility of cadmium in a marsh-cove ecosystem and the relation to cadmium concentration in biota. *Environ. Health Perspect.* **28**: 67–73.

Knutson, A. B., P. L. Klerks, and J. S. Levinton. 1987. The fate of metal-contaminated sediments in Foundry Cove, New York. *Environ. Pollut.* **45**: 291–304.

Lavie, B., and E. Nevo. 1982. Heavy metal selection of phosphoglucose isomerase allozymes in marine gastropods. *Mar. Biol.* **71**: 17–22.

Lavie, B., and E. Nevo. 1986. The interactive effects of cadmium and mercury pollution on allozyme polymorphisms in the marine gastropod *Cerithium scabridum. Mar. Pollut. Bull.* **17**: 21–23.

LeBlanc, G. A. 1982. Laboratory investigation into the development of resistance in *Daphnia magna* (Straus) to environmental pollutants. *Environ. Pollut. Ser. A Ecol. Biol.* **27**: 309–322.

Levinton, J. S. 1980. Genetic divergence in estuaries. Pp. 509–520 in *Estuarine Perspectives,* V. S. Kennedy, ed. Academic Press, New York.

Macnair, M. R. 1987. Heavy metal tolerance in plants: a model evolutionary system. *Trends Ecol. Evol.* **2**: 354–359.

McNeilly, T. 1968. Evolution in closely adjacent plant populations. III. *Agrostis tenuis* on a small copper mine. *Heridity* **23**: 99–108.

McNeilly, T., and A. D. Bradshaw. 1968. Evolutionary processes in populations of copper-tolerant *Agrostis tenuis* Sibth. *Evolution* **22**: 108–118.

Moraitou-Apostolopoulou, M., M. Kiortsis, V. Verriopoulos, and S. Platanistioti. 1983. Effects of copper sulphate on *Tisbe holothuriae* Humes (copepoda) and development of tolerance to copper. *Hydrobiologia* **99**: 145–150.

Nevo, E., R. Ben-Shlomo, and B. Lavie. 1984. Mercury selection of allozymes in marine organisms: prediction and verification in nature. *Proc. Natl. Acad. Sci. USA* **81**: 1258–1259.

Rahel, F. 1981. Selection for zinc tolerance in fish: results from laboratory and wild populations. *Trans. Am. Fish. Soc.* **110**: 19–28.

Resource Engineering. 1983. *Preliminary Site Background Data Analysis of Foundry Cove.* Resource Engineering, Houston.

Sokal, R. R., and F. J. Rohlf. 1981. *Biometry.* Freeman, San Francisco.